Regional Operational Plan CF.4K.2014.23

Kodiak Management Area Pacific Herring Hydroacoustic Assessment, 2014

by

Michelle L. Moore

May 2014

Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

Symbols and Abbreviations

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in the following reports by the Divisions of Sport Fish and of Commercial Fisheries: Fishery Manuscripts, Fishery Data Series Reports, Fishery Management Reports, and Special Publications. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figure or figure captions.

Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

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Michelle L. Moore

Alaska Department of Fish and Game, Division of Commercial Fisheries, Kodiak

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Division of Commercial Fisheries

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SIGNATURE/TITLE PAGE

Project Title:	Kodiak Management Area Pacific Herring Hydroacoustic Assessment
Project Leader(s):	M. Birch Foster, Fishery Biologist III Michelle L. Moore, Fishery Biologist II
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Project Nomenclature:	
Period Covered:	Spring 2014
Field Dates:	April 1–May 31
Plan Type:	Category I

Approval

Title	Name	Signature	Date
Project Leader	M. Birch Foster	M. Birch Foster	5/12/14
Project Leader	Michelle Moore	Michelle Moore	5/12/14
Research Coordinator	Nick Sagalkin	Nick Sagalkin	4/25/2014

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ABSTRACT

This report specifies the methods and procedures that the Alaska Department of Fish and Game (ADF&G) uses for conducting Pacific herring *Clupea pallasi* hydroacoustic surveys in the Kodiak Management Area (KMA). Abundance and distribution of known herring concentrations in select bays will be analyzed using single-beam or split beam hydroacoustic transducers. Acoustic signatures will be recorded and analyzed after completion of the hydroacoustic surveys. A small specially constructed mid-water research trawl will be used to verify species and to collect small samples from which biological characteristics will be obtained. In addition, herring samples may be tested for *Ichthyophonus hoferi*, North American Viral Hemorrhagic Septicemia Virus, and Erythrocytic Necrosis Virus. Hydroacoustic surveys are typically conducted in April.

Key words: Pacific herring, *Clupea pallasi*, hydroacoustics, survey, Kodiak

PURPOSE

The purpose of this project is to increase our knowledge and understanding about the population size, distribution, and health of discrete spawning aggregates of Pacific herring (*Clupea pallasi*) in select Kodiak Management Area (KMA) bays, and to develop hydroacoustic survey methods and techniques.

BACKGROUND

As one of the most abundant species in the North Pacific, herring are extremely important for commercial and subsistence fishing, and as prey for fish, birds, and mammals. The earliest recorded commercial herring harvest in the Kodiak area occurred in 1912 when herring were harvested primarily for food, bait, fishmeal, or oil. During the peak years of reduction fisheries (1934–1950), the average annual harvest was 31,600 tons (Reid 1971). The Alaska Department of Fish and Game (ADF&G) KMA has supported a sizeable commercial sac roe fishery since 1964 (Spalinger 2014a). Pacific herring are also harvested by Kodiak residents for subsistence purposes. Subsistence catches are relatively small (from 2002–2011 the annual total Kodiak area harvest averaged less than 2 tons; Spalinger 2014a), though lack of reporting may hinder complete assessment of local use of Pacific herring.

The ADF&G manages both the KMA sac roe and food and bait herring fisheries. Currently, for both types of commercial fisheries, guideline harvest levels (GHLs) are formulated for individual harvest areas, which are designated based on the department's assessment of individual spawning areas or specific harvest areas. Establishing GHLs involves evaluation of a variety of information regarding stock status trends, including the following indicators 1) fishery performance during preceding season or seasons (i.e., harvest timing, harvest duration, average school size, and fishermen's estimates of available biomass), 2) trends in age composition (i.e., the proportion of age-2 fish in the spawning biomass, the level of recruitment (age 3) in catch samples, and the proportion of the spawning population age 5 and younger), and 3) observations of spawning and juvenile herring.

Aerial and hydroacoustic surveys are used to assess Pacific herring biomass; however, to date these techniques have provided only a limited evaluation of stock strength. Problems associated with aerial and sonar stock assessment in the KMA exist for numerous reasons 1) the KMA encompasses a large geographical area that comprises many separate and distinct concentration areas (e.g., bays), 2) herring are visible from the air only when near the surface; the deep turbid bays of Kodiak limit the time fish are observable from the air, 3) adverse weather conditions, 4) distinct schools of herring move in and out of the observed areas, so that biomass estimates may

be duplicated, incomplete, or incorrectly assigned to a spawning stock location, and 5) various KMA herring stocks have different spawn timing. "Miles of spawn" (basically quantifying miles of spawn on shorelines using aerial surveys) methods do not work in Kodiak as there appears to be a significant amount of subtidal spawning occurring too deep (10 to 20 fathoms) to detect from aerial surveys. Age-structured-analysis (ASA) modeling has been attempted, but has been limited by lack of good biomass estimates to "anchor" the model.

Herring stocks throughout the North Pacific are sampled for pathogens including *Ichthyophonus hoferi* (Ichthyophonus), Erythrocytic Necrosis Virus (VENV), and North American Viral Hemorrhagic Septicemia Virus (NA-VHSV). Ichthyophonus is a protozoan that can be highly pathogenic to Pacific herring. While no natural epizootics have been reported to date in Pacific herring, Ichthyophonus has been associated with population decline in Atlantic herring populations (Daniel 1933; Sindermann 1958; Mellergaard and Spanggaard 1997). From 2007–2008, herring were sampled from several stocks in the KMA. In 2007, 47 percent of sampled herring in February, and 43 percent of sampled herring from April in Uganik Bay tested positive for Ichthyophonus (M. Birch Foster, 2007, unpublished manuscript, Alaska Department of Fish and Game, Kodiak, Alaska). Ichthyophonus was also present in other discrete stocks, but in much lower percentages. When possible, herring will also be sampled for two viruses, VENV and NA-VHSV.

We propose to conduct opportunistic hydroacoustic surveys to quantitatively assess the spawning biomass of Pacific herring in the waters surrounding Kodiak Island. This operational plan outlines the application of hydroacoustic techniques to assess the distribution, abundance, and biological attributes of Pacific herring found in large aggregations in various bays and inlets around Kodiak Island. Associations and foraging activity of Steller sea lions *Eumetopias jubatus* and humpback whales *Megaptera novaeangliae* will also be documented.

OBJECTIVES

There are four primary objectives to this project:

1. Locate and quantify biomass of large aggregations of Pacific herring within the KMA prior to or during spring spawning.
2. Collect biological samples of the surveyed populations to estimate age, size, and target strength.
3. Collect biological samples of populations to test for *Ichthyophonus hoferi*, North American Viral Hemorrhagic Septicemia Virus, and Erythrocytic Necrosis Virus.
4. Document abundance and locations of marine mammals in association with herring aggregations.

METHODS

Study Area

The KMA comprises the waters of the Kodiak Archipelago and that portion of the Alaska Peninsula extending from Cape Douglas southwest to Kilokak Rocks (Figure 1). The archipelago is approximately 250 kilometers (150 miles) long, extending from Shuyak Island in the north to the Trinity Islands in the south. The Alaska Peninsula portion of the KMA is about 267 kilometers (160 miles) long and is separated from the archipelago by Shelikof Strait (Figure 1).

The KMA is divided into 13 districts that define geographical areas used to manage both herring sac roe and food and bait fisheries (Figure 2). For the sac roe fishery, each district is divided into sections that define the spawning area used by specific herring stocks or a geographical area (Spalinger 2014b).

Hydroacoustic survey location goals for 2014 in order of priority are Kukak, Danger, and Uganik bays based on the large consistent aggregations found in these areas (Figure 3).

Survey Methods

In 2014, hydroacoustic surveys will be conducted in April aboard either the ADF&G research vessel R/V *Resolution* or R/V *K-Hi-C*. Shipboard sonar equipment will always be used, and when possible a side-deployed transducer (Figure 4) will be used for duplication of effort using different hydroacoustic frequencies.

The basic hydroacoustic assessment consists of a four-stage sampling approach (Cochran 1977) 1) reconnaissance, 2) verification, 3) repeated echointegrations 4) and subsampling school groups for biological information.

Prior to a Survey

The first step in the sampling approach, aerial or sonar reconnaissance, is to locate areas of herring aggregations. This stage is assisted by historical information and local fishers' knowledge of the site fidelity of spawning or staging herring.

In addition, predator assemblages (primarily Steller sea lions, humpback whales, and various seabirds) aggregate in areas where herring are present, which assists in locating school groups. Associations and foraging activity of these predators with the herring concentrations will be documented (Thorne et al. 2003). A detailed log of marine mammal observations will be recorded. When possible, whale fluke identification digital pictures and sea lion haul outs will be recorded to document abundance estimates. A project leader (Foster) is authorized to pursue whales under the authority of the NOAA permit (#14296) for photo identification purposes.

Water quality (temperature, salinity, and pH) will be measured, at a depth of approximately 2 meters, and recorded into the field notebook. Water quality will be examined using a YSI 85-D analyzer. The measured variables will used in post-processing to correct for the speed of sound in water.

Survey

The second stage of the survey is to verify the presence of herring in suspected areas and develop a map of the area occupied by the school-group using a searchlight sonar, echosounder, and GPS plotter. An infrared underwater camera may be used to verify species identification and characterize general school dispersion. Net or jig sampling may also be used to verify species.

The third stage of the survey is echointegration. Once the boundaries of the herring distribution are established (from the second stage), an echointegration survey (Thorne 1983a,b; MacLennan and Simmonds 1992) is conducted to estimate the fish density. Two different echo integration systems may be used during surveys. A BioSonics DT-X (70 kHz single beam towable; Appendix A1–A2; Biosonics 2009) and Simrad EK-60 (split beam 38 and 200 kHz inhull; Simrad 2003) will be used in tandem, when possible, for duplication of effort and to ensure accuracy. Instructions for setting up the BioSonics unit are displayed in Appendices A1–A2. Transects are made following a zigzag survey track (Sigler and Csepp 2006; Figure 5). The

'zigs' are separated from the 'zags' to create two series of approximately orthogonal transects and to eliminate autocorrelation (Thorne 1983a). The sonar remains in continuous operation during the echo integration measurement phase of the survey 1) to evaluate the possibility of school avoidance to the vessel, 2) to monitor the school-group until the end of the transect, and 3) to avoid collision with submerged rocks and the shoreline. All echo integration systems will be calibrated prior to the survey using a known-density tungsten sphere and resultant target strength (-db) will be documented. More information pertaining to calibration of acoustic gear can be found in Foote et al. (1987).

Post Survey

The fourth and final stage of the herring survey is to sample the herring schools for biological information. Live-capture sampling will be conducted using a custom designed mid-water research trawl when aboard the R/V *Resolution* to sample the previously echo integrated fish concentrations for biological information. The cod end of the net will be emptied on deck where a minimum of 240 herring will be saved for total length and weight sampling upon return to Kodiak. Samples will be immediately double bagged in polyethylene bags and tagged with location, date, time, and frozen. Additional herring may be bagged for pathogen sampling on the boat or upon return to Kodiak. These samples must not be frozen. Pathogen sampling procedures are found in Appendix B.

Weight (g) and length (total length in mm) of the herring will be measured and age will be determined by examining scales. The length information will be used to establish the target strength in order to calculate biomass estimates.

Target strength information is required to scale acoustic data to absolute abundance. Historically, estimates of Pacific herring biomass have been based on a target strength to fish body length relationship for Pacific herring of $TS = 26.5 \times \log L - 76.4$, where L is length in cm (total length). This equation evolved from many different sources and in-situ experiments (Thorne 1977; Trumble et al. 1982), but has recently been refined (Ona 2003; Gauthier and Horne 2004; Stokesbury et al. 2000; Thomas et al. 2002). In summary, the original target strength theories are reasonable, but have been adapted to incorporate changes associated with both depth and season. Target strength will be estimated using the methods described by Gauthier and Horne (2004) with consideration given to season and depth of fish surveyed.

Biomass estimates (mean density of transects extrapolated to surface area surveyed) will then be calculated using Echoview software. The repeated survey estimates are used to determine the precision of the biomass estimates (Scheaffer et al. 1986); naturally variability within individual surveys will be assessed as well. Many times the herring can move in or out of survey areas and every attempt to create robust but repeatable survey tracks will be made.

SCHEDULE AND DELIVERABLES

Results of the spring hydroacoustic survey will be reported as soon as possible in memo format or verbally to management staff inseason as needed. In addition a regional information report will be authored by Michelle Moore.

Date	Activity
April 1–May 15	Survey and sampling occur
Fall 2014	Data analysis
Winter 2014	Report written and published

RESPONSIBILITIES

The project leaders are M. Birch Foster and Michelle Moore. They will work jointly to collect and analyze hydroacoustic data. They will coordinate with both the skippers of the R/V *Resolution* and R/V *K-Hi-C* in locating and sampling aggregations of herring during the spring spawning season. The project leaders will also work with the herring management biologists to assist with biomass estimation as needed during the sac roe fishery.

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FIGURES

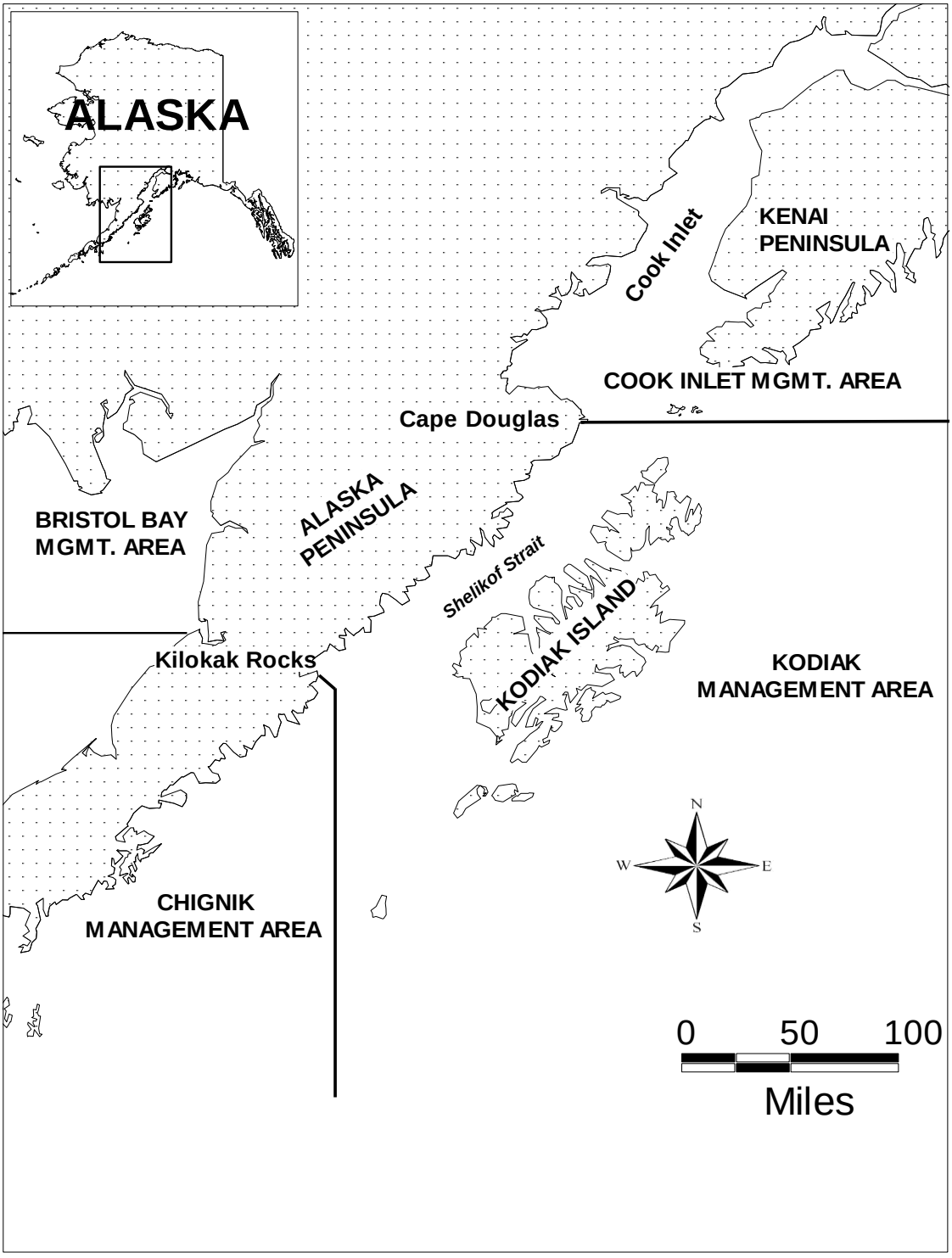

Figure 1.–Map of the Kodiak Management Area and surrounding management areas.

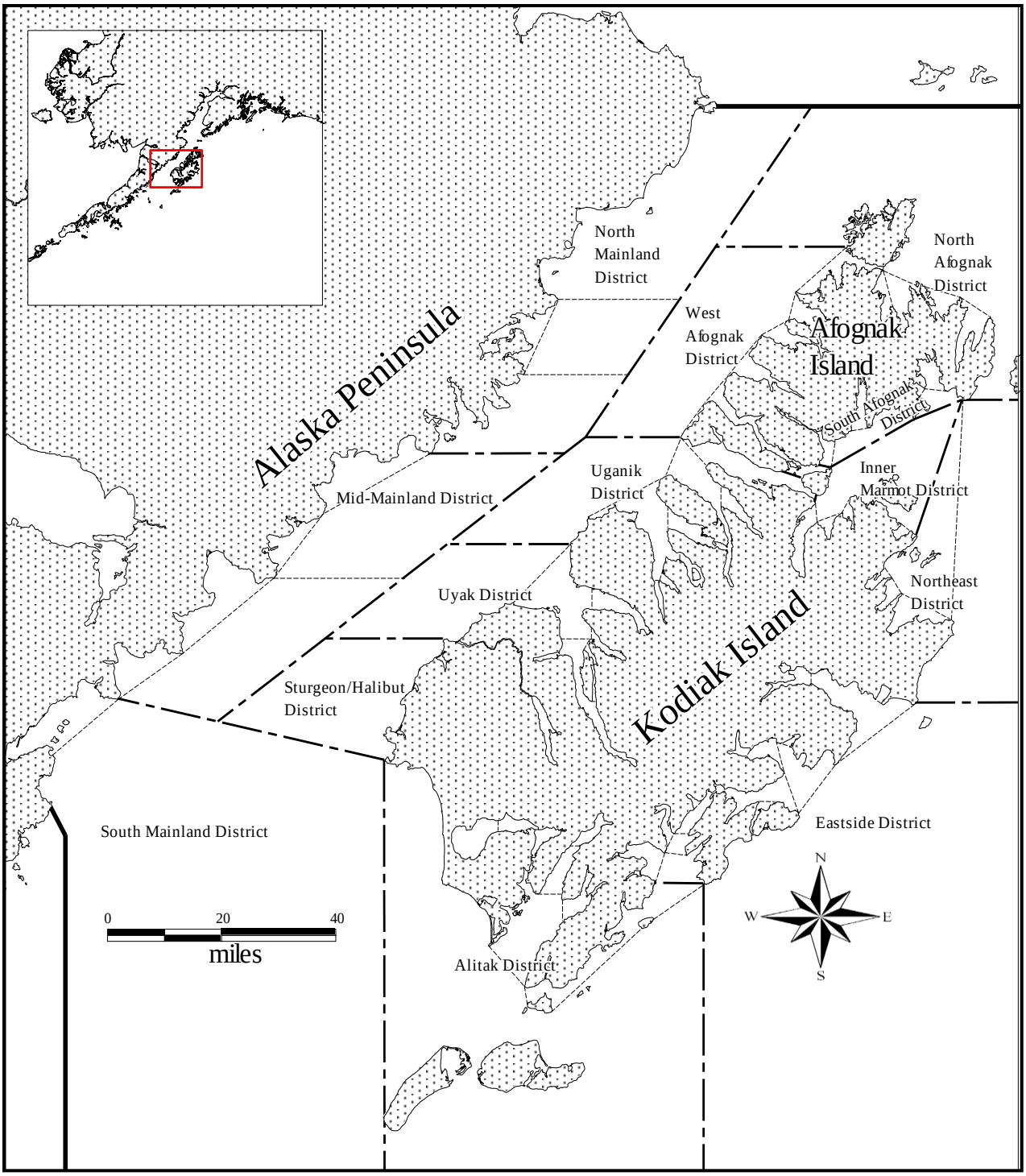

Figure 2.–Map depicting the commercial herring fishery districts for the Kodiak Management Area.

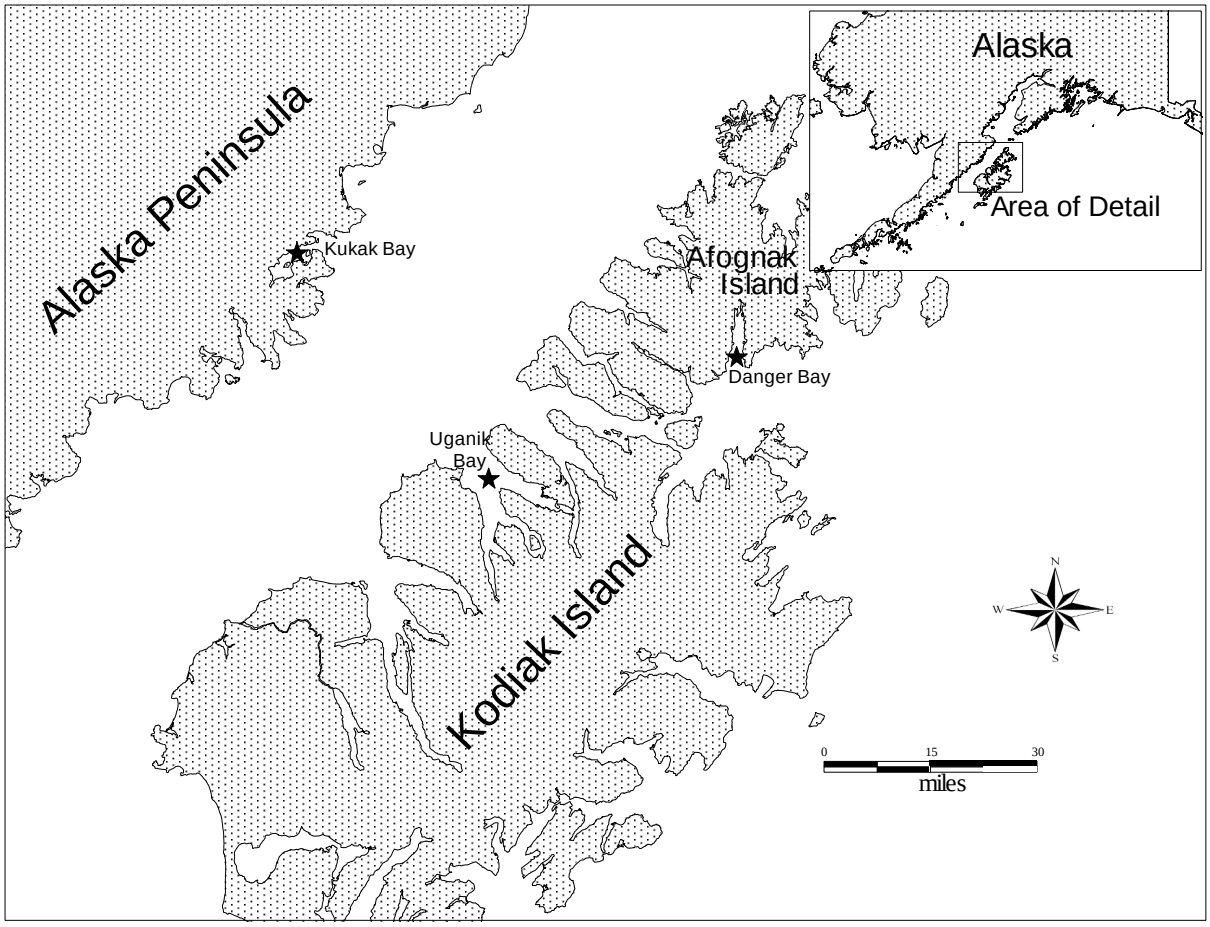

Figure 3.–Map of a portion of the KMA, showing the preferred hydroacoustic survey locations.

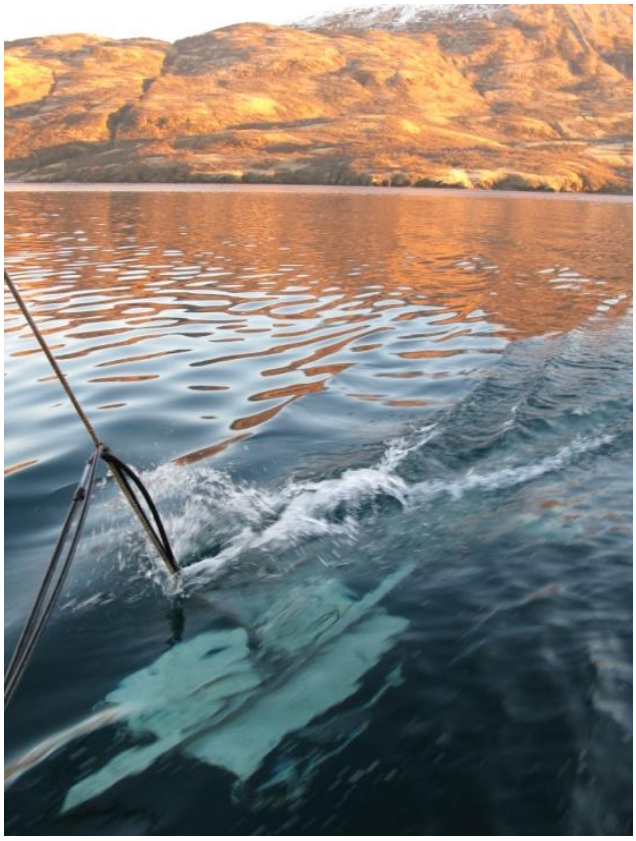

Figure 4.–The side-deployed tow fin with attached transducer.

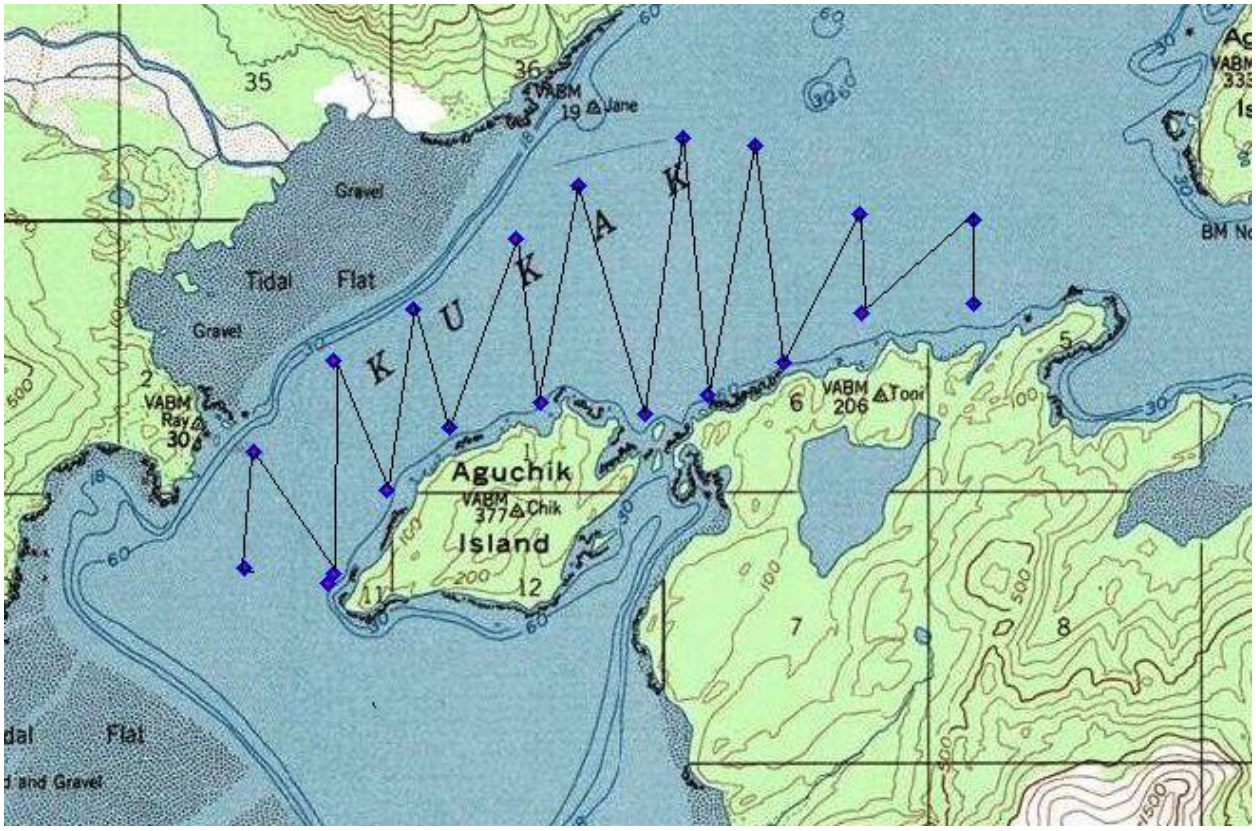

Figure 5.–Example of zigzag survey track from Kukak Bay.

APPENDIX A. BIOSONICS HYDROACOUSTIC SYSTEM

Appendix A1.–Configuration of the Biosonics hydroacoustic transducer, cable, DT-X controller, GPS, and computer.

The Biosonics DT-X system collects data from a number of components and sends that data to a computer for visualizing and recording. Hydroacoustic information is collected by the echosounder's transducer while spatial information is collected via a GPS antenna, and the resulting spatially and temporally correlated echogram is displayed by the program Visual Acquisition. This appendix describes how to connect the hardware required for hydroacoustics using the Biosonics DT-X system.

A number of components will be assembled to collect the hydroacoustic and position data. A GPS receiver antenna (Figure A1.1) is mounted in a high location on the vessel with an unobstructed view of the sky, and the cable is routed into the vessel's cabin to the Biosonics DT-X surface unit housed in a Pelican case.

Figure A1.1.–GPS receiver antenna for Biosonics DT-X hydroacoustic system.

The hydroacoustic transducer is mounted on a tow sled or on the end of a pole attached to the vessel's gunnel (Figure A1.2). The rubber-coated ceramic face is extremely sensitive and must not be damaged in any way. Oils and other products should not be allowed to come in contact with the transducer face as these will change the acoustic interface with the water and provide inaccurate data. The transducer data cable (Figure A1.2) is attached via a waterproof connector to the transducer. Silicone dielectric grease or an electronics-safe water dispersion spray lubricant such as silicone or Corrosion Block (Midwest Corrosion Products, Lancing MI) should be used inside the plug interface. The outside of the connection can be wrapped in electrical tape for extended deployments such as when mounted on the transducer pole. The end of the transducer cable with the green metal military-style connector is routed into the vessel's cabin to the Biosonics DT-X surface unit.

-continued-

Figure A1.2.–DT-X transducer and cable.

The Biosonics DT-X surface unit (Figure A1.3) is placed on a flat surface using restraints or a grip pad, in a dry location on the vessel.

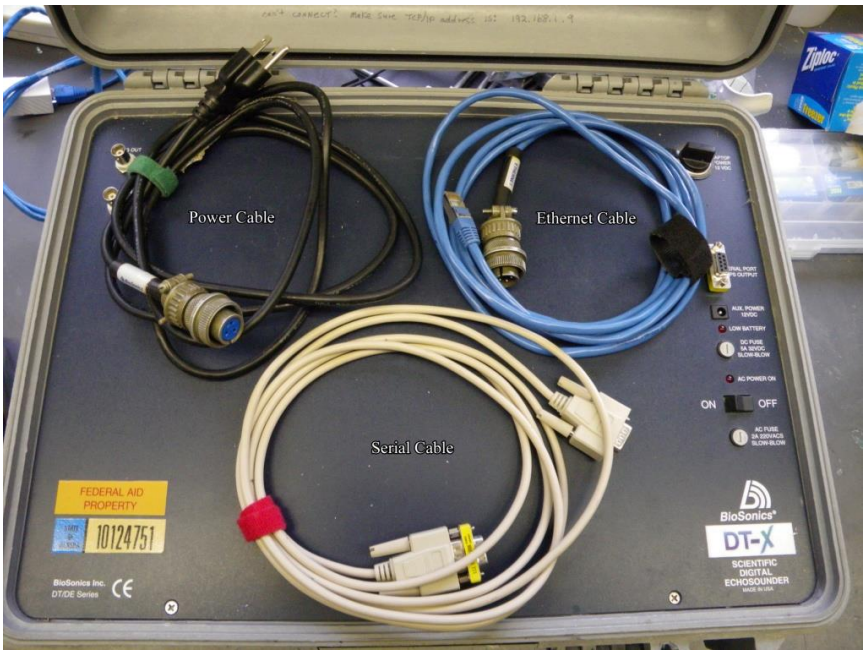

Figure A1.3.–Biosonics DT-X surface unit.

On the back and side panels of the Biosonics DT-X surface unit are a number of military-spec waterproof electronics plugs. All the cables that will attach to these plugs are unique so there is no danger of attaching the wrong cable to the wrong plug. The four connections (Figure A1.4) that will be made to the DT-X Pelican case are

1. 110v AC cable to power supply
2. GPS data cable to GPS receiver

-continued-

3. Echosounder data cable to transducer
4. Ethernet cable to computer

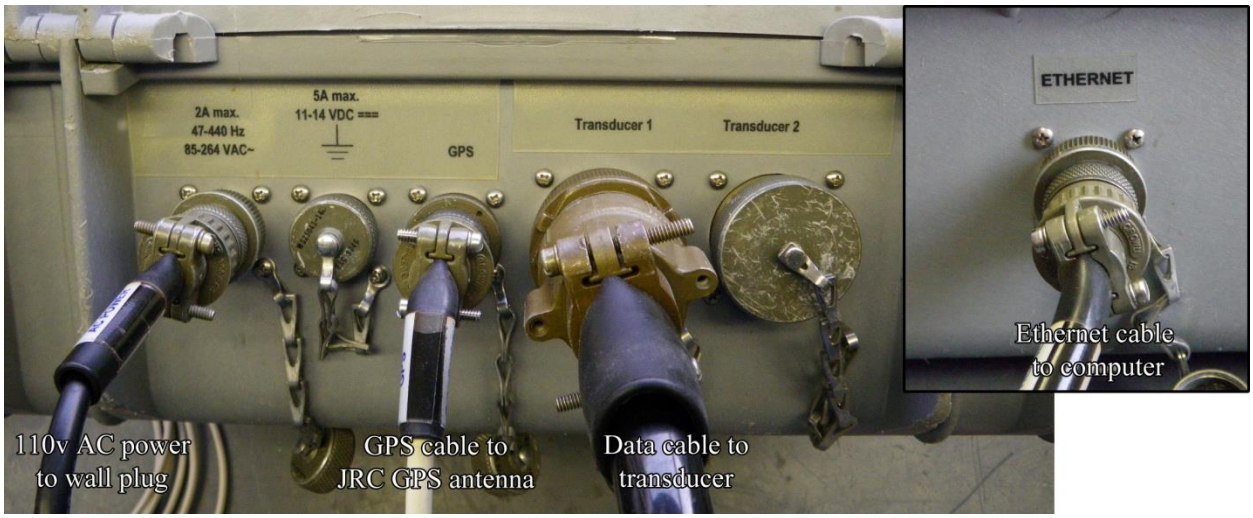

Figure A1.4.–Biosonics DT-X control module cable connections

All military-spec connectors should be fully seated with the threaded collar turned fully clockwise to tighten, and then backed off a quarter turn. It is not necessary or desirable to have these connectors made overly tight. The Ethernet cable from the Biosonics DT-X surface unit is connected to the Ethernet port on a computer. The serial connection can be used to send a GPS NMEA data stream to another program in the computer if that becomes necessary.

Appendix A2.–Use of Biosonic's Visual Acquisition to record and view the echogram.

The interface used to view and log the echogram created by the hydroacoustic system is a program called Visual Acquisition (Figure A2.1). It uses a real-time, side-scroll view to display the echogram much like a shipboard downsounder. In the program, the operator can set the environmental parameters such as water temperature and salinity (which affect the speed of sound in water), file naming and recording protocols for logging the echogram, and many other settings which affect how the echogram is viewed and what data is logged.

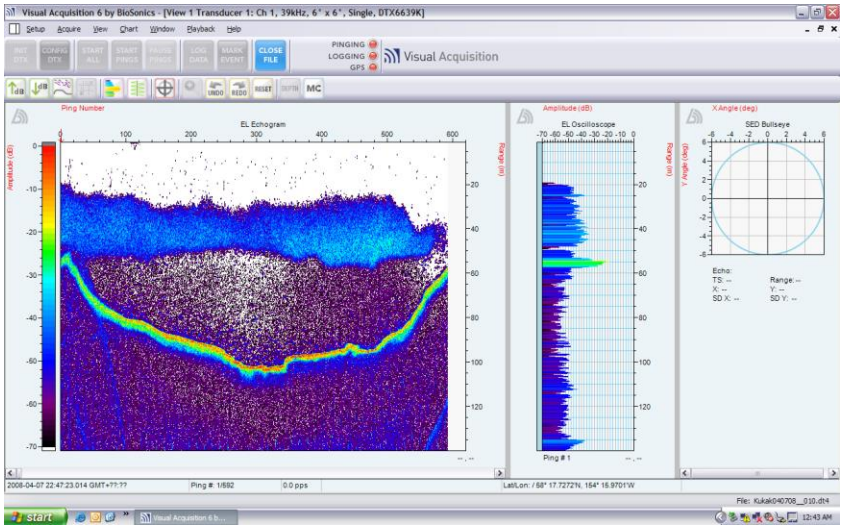

Figure A2.1.–Screen shot of a school of herring in Visual Acquisition 6.

Outlined below are the specific steps used to initialize, view, log, and shut down the Visual Acquisition environment. More comprehensive information about the program can be found in the Visual Acquisition User Guide. The hydroacoustic transceiver unit ***MUST*** be submerged in water before pinging can be initiated or irreparable damage will occur to the ceramic element in the unit. Before initializing the unit, a temperature, salinity, and pH reading of the water should be taken using the YSI 85-D sensor (Figure A2.2). Press the ON/OFF button to power the unit, and suspend the weighted electrode at the end of the unit's cable over the side of the vessel and allow it to sink to approximately 2 m depth. Take salinity and temperature readings and record these in the survey logbook.

Figure A2.2.–YSI 85-D

-continued-

1. Check all the cable connections including the
    a. power,
    b. GPS cable to the GPS antenna,
    c. cable to the transducer,
    d. Ethernet cable to the computer.
2. On the laptop computer, check the IP address to ensure it will receive data from the DT-X unit.
    a. Go to *Start > Settings > Control Panel > Network Connections > Local Area Connections*
    b. Under the *General* tab, under *This connection uses the following items:* click the *Internet Protocol (TCP/IP)* item
    c. Click the *Properties* button
    d. Select the *Use the following IP address* radio button
    e. In the *IP address* field, type *192.168.1.1*
    f. In the *Subnet mask* field, type *255.255.255.0*
    g. Click OK and close the control panel.
3. Turn on the power switch in the DT-X control module Pelican case (a red LED will illuminate). After about 20 seconds a beep will sound.

Start the Visual Acquisition 6 software; *Start > Programs > Biosonics > Visual Acquisition 6 > Visual Acquisition 6*.

1. Click on the *INIT DT-X* button. This will start the GPS feed and allow the unit to interrogate the transducer. A series of tones will sound.
2. A *System Information* window will appear which shows the status of various components. Click OK.
3. Click the *CONFIG DT-X* button.
4. The *Configure Echosounder* window opens.
5. You will now enter several parameters. In the *Data Collection Parameters* box, enter 0 and XX m as the start and end range (depends on the depth of the deepest point in survey). The Threshold Level (dB) should be set to -70. In the *Pulse Control* box, set the Pulse Rate (pps) to 1.0 (or possibly slower) and the Pulse Duration (ms) to 1.0.
6. Under the *Transducer 1* item in the hierarchical list, select the *Environment* item.
7. Enter the water temperature and salinity collected with the YSI meter, then click the *Compute…* button.
8. Select the *Data Logging* item and change the File Prefix to the area and the survey day, all separated by an underscore (e.g., Kukak04_11_2014).
9. Click OK to close the *System Information* window.

-continued-

10. Click the *START PINGS* button to begin viewing the echogram on the screen (but not record the data), or click *START ALL* to view the echogram AND begin logging the data to hard drive. If you are logging data, note the file name being written to the drive in the lower right-hand corner of the window and write this in the logbook.
11. To stop logging data, click the *CLOSE LOG* button, or to close the log AND stop pinging entirely, click the *END ALL* button.

In summary settings should be:

- Transmit/Receive
    - Active Transmission
    - Transmit Pulse Duration (ms): 1.0
    - Start Range (m): 0
    - End Range (m): XX
    - Calibration Correction (dB): 0
    - Data Collection Threshold Level (dB): -70
- Sensors/Mounting
    - All default values used
- Environment
    - Temperature (°C): *Set based on measured value*
    - Fresh Water: No
    - Salinity: *Set based on measured value*
    - Reference Depth (m): 2
    - pH: *Set based on measured value*
- Bottom Detection
    - Turned off
- Echo Detection
    - Turned off
- Data Logging
    - File duration(mins): 30
    - File Prefix: Use the survey areamonth _day_year (e.g., Kukak4_11_2014)
    - File Suffix: leave blank
    - Logging Folder: C:\Biosonics\VisualAcquisition6\data

-continued-

- File Cutting Mode: Elapsed Time
- File Naming Mode: Timestamped
- Create DT4 Files: Yes
- Create HAC Files: No
- Create DTB Files: Yes

IMPORTANT: Make sure the program Visual Acquisition stops pinging before turning off the power to the DT-X surface unit.

With the unit actively pinging, a number of settings can be varied to change the way the data is displayed. These settings can be changed via using function keys or in some cases buttons in the Chart Toolbar.

- Pressing F1 at any time brings up the electronic .pdf Visual Acquisition 6 manual.
- Pressing F3 (or using the toolbar button) increases the display threshold and applies a higher (stronger) echo threshold to displayed data, which effectively reduces noise and filters out weaker ping returns. If there is a lot of feed or debris in the water, this can reduce clutter and make fish more apparent. It also "shrinks" the fish echoes and makes them fainter. Note that this only affects the displayed echogram. The actual threshold of the recorded data being logged is set in the System Information window when the system is configured.
- Pressing Shift + F3 (or using the toolbar button) decreases the display threshold and does the opposite of decreasing the threshold.

APPENDIX B. SAMPLING FOR PATHOGENS

Appendix B1.–*Ichthyophonus hoferi* sampling.

Ichthyophonus sampling- adult and juvenile fish tissue for explant cultures- 60 fish

1. Disinfect the outside of the fish with 100 ppm Iodophor solution and dry with a paper towel.

2. Aseptically remove the heart using disinfected forceps and scalpel. Be careful not to introduce contaminating organisms (bacteria from the outside of the fish or intestinal tract for example) to the sample tissues. Place into a sterile Whirl-pak bag and refrigerate.

3. To avoid cross-contamination, disinfect sampling utensils between fish by removing organic material, soaking in 100 ppm iodophor solution, and rinsing with clean water before reuse. You can also use alcohol and flaming to sterilize instruments between samples. Alternately, disposable utensils can be used (sterile single edge razor blades and sterile tongue depressor wooden sticks) and thrown out after collection of a single sample.

4. DO NOT FREEZE. Bagged tissue samples should be placed into a cooler or insulated container and sent ASAP to the fish pathology laboratory for processing. Samples must be kept cold in transit. Be sure the samples are not in direct contact with gel ice to prevent freezing.

Kidney/Spleen pools for VHSV- 60 fish

1. Disinfect the outside of the fish with 100 ppm Iodophor solution and dry with a paper towel.

2. Aseptically remove the kidney and spleen using disinfected forceps and scalpel (Figure 1). If the amount of tissue is very small (ie less than 1 g) a piece of liver may also be included. Be careful not to introduce contaminating organisms (bacteria from the outside of the fish or intestinal tract for example) to the sample tissues. Place the kidney and spleen from each fish into a single sterile, numbered Whirl-pak bag and refrigerate.

3. To avoid cross-contamination, disinfect sampling utensils between fish by removing organic material, soaking in 100 ppm iodophor solution, and rinsing with clean water before reuse. You can also use alcohol and flaming to sterilize instruments between samples. Alternately, disposable utensils can be used (sterile single edge razor blades and sterile tongue depressor wooden sticks) and thrown out after collection of a single sample.

4. DO NOT FREEZE. Bagged tissue samples should be placed into a cooler or insulated container and sent ASAP to the fish pathology laboratory for processing. Samples must be kept cold in transit. Be sure the samples are not in direct contact with gel ice to prevent freezing.

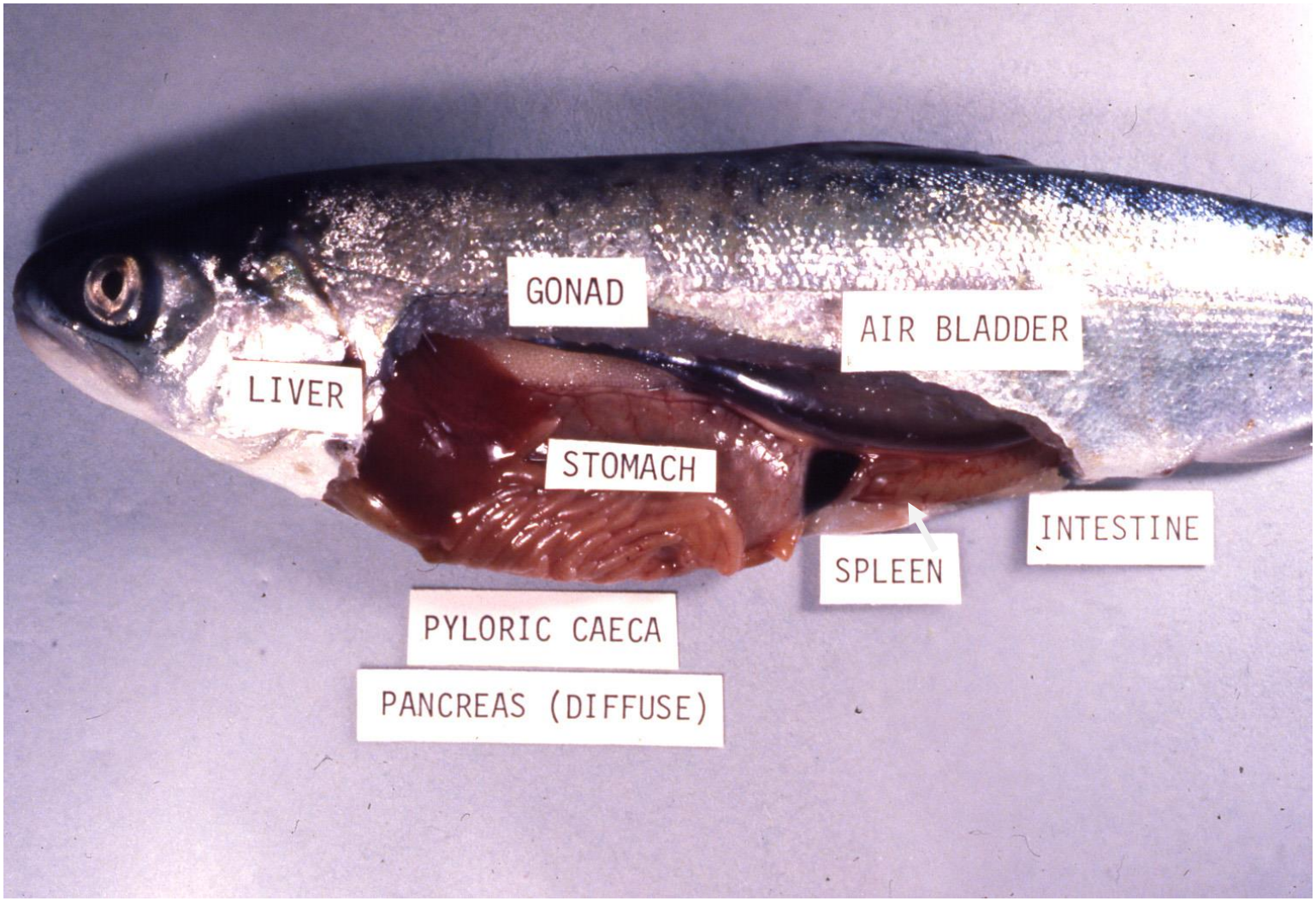

Figure 1.–Fish partially dissected with the spleen labeled.

Erythrocytic Necrosis Virus (VENV) Sampling**:**

1. On slide "A" express a (small) drop of blood or hemolymph about one-half inch from the end.
2. The edge of a second slide "B" is placed on the surface of slide "A" at about a 45° angle and is moved to the right until contact with the drop of blood.
3. Contact with the blood will cause the drop to spread along the edge of slide "B" due to capillarity. Slide “B" is then pushed to the left, being careful to keep the edge pressed uniformly against the surface of slide "A".
4. The size of the drop of blood and acuteness of the angle formed between the slides will determine the thickness of the film, A more acute angle results in a thicker film. Less thick is better.
5. The smear is allowed to air dry for transpor t in a slide box and later staining.

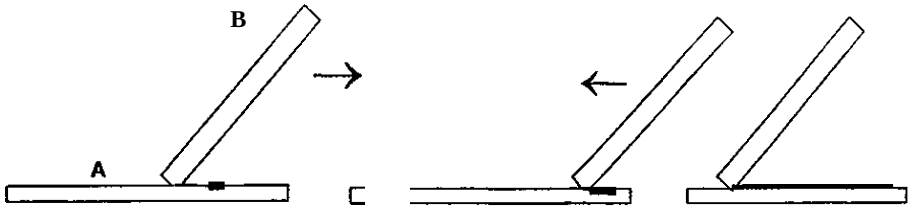

Figure 1.–Preparation of a thin blood smear.

Appendix B4.–Sample Submission Form – ADF&G Fish Pathology Laboratory.

Date COLLECTED: _____

Facility/contact person:_____

Address and email: _____

Lot (BROOD YEAR/STOCK/SPECIES):_____

Life stage:_____

Sex if applicable:_____

Date outbreak noticed:_____

Problem history:_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Recent medications: _____

Are these samples an FTP requirement? YES___ NO ___

If yes, what is the FTP number? _____